# Lab 6:
# A Kinetics Experiment of the Reaction of Coumarin-102 with Base

FALL 2021

CHEM 125 : GENERAL CHEMISTRY LAB (II)

THE UNIVERSITY OF ILLINOIS AT CHICAGO

UIC

# Experimental Objectives

**Determine rate law for the reaction Using Fluorescence:**

*Coumarin-102 + $OH^-$ → Coumaric Acid*
*(fluorescent species) (non-fluorescent species)*

**Rate of rxn = k[C102]$^a$[NaOH]$^b$**

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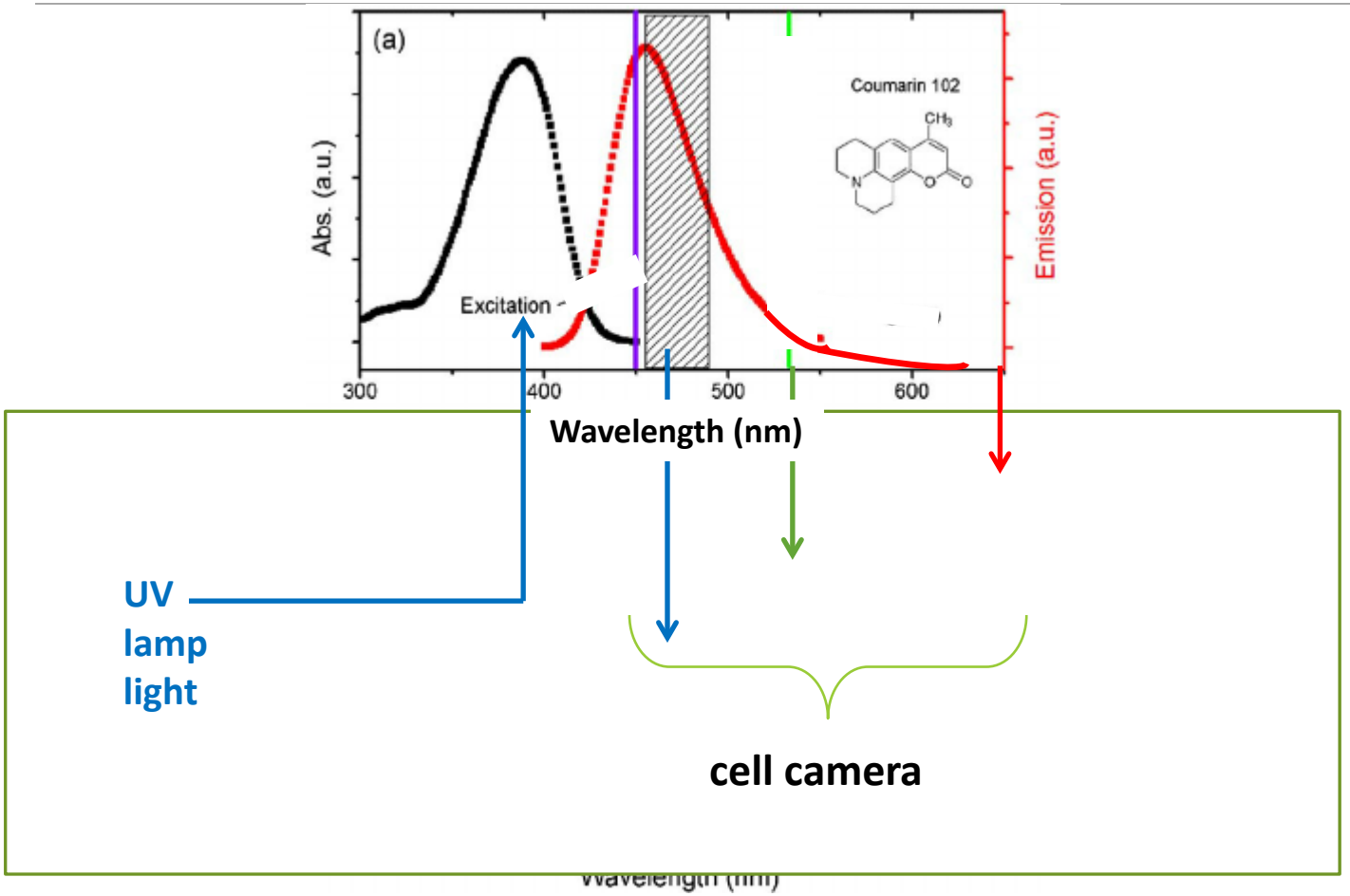
*Need to determine orders of rxn, a and b*

*Further general information on kinetics studies of chemical reactions can be found in textbook "Chemistry The Molecular Nature of Matter and Change, Chapter 16, especially Section 4 on Integrated Rate Laws.*

# Background Information:
# What is fluorescence?

## Emission of Light Resulting from Absorption of Light
## For molecules, wavelength of fluorescence longer than that of absorption

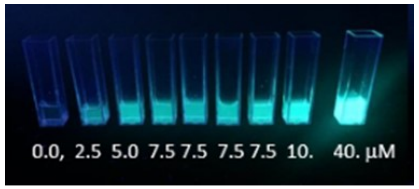


# Experimental Approach (1 of 2)

**In-Lab session: Parts I and II (Overview)**

**Part I: Check how C-102 Fluorescence relates to Concentration**

Make up series of solution concentrations & measure relative fluorescence intensity with cell phone camera using ImageJ to quantify intensity. Plot fluorescence intensity, I, versus [C-102] to show linearity.




ImageJ can be downloaded from:
https://imagej.nih.gov/ij/download.html

YouTube Link for using of ImageJ
https://www.youtube.com/watch?v=cStjSTRnuBA

**See auxiliary handout "How to use ImageJ" on downloading and using ImageJ for more details.**

**Part II: Determine Order of [C-102]** , a, in Rate law  Rate = $k[\text{C-102}]^a[\text{NaOH}]^b$

Run reaction with fixed, excess [NaOH].

Then  **Rate = $k[\text{C-102}]^a[\sim\text{constant}]^b = k'[\text{C-102}]^a$**

Fit data as relative fluorescence intensity, $I/I_0$ to functions forms of 0th order, 1st order, and 2nd order integrated rate laws.

*(***Note: $I_0$ is a reference intensity NOT intensity at t=0. It is fluorescence from the cuvette without NaOH whereas I is fluorescence intensity from cuvettes with NaOH.)*

| **0th Order:** | **1st Order:** | **2nd Order:** |
|---|---|---|
| **$(I/I_o)$ vs time** | **$\ln(I/I_o)$ vs time** | **$1/(I/I_o)$ vs time** |

# Summary of Integrated Rate Laws in Determining Order in C102 (Part II)

| Order | Rate Law (with excess [NaOH]) | Integrated Rate Law | Slope | Units for k' |
|---|---|---|---|---|
| If $0^{th}$ order in [C102] | Rate=k'$[C102]^0$ = k' | $[C102]_{t=t} = [C102]_{t=0} - kt$ ($I/I_o$) versus time will be linear | -k' | M/s |
| If $1^{st}$ order in [C102] | Rate = k'[C102] | $\ln([C102]_{t=t}/[C102]_{t=0}) = -kt$ $\ln(I/I_o)$ versus time will be linear | -k' | 1/s |
| If $2^{nd}$ order in [C102] | Rate = k'$[C102]^2$ | $1/([C102]_{t=t} - 1/[C102]_{t=0}) = kt$ $1/(I/I_o)$ versus time will be linear | k' | 1/M·s |

***Remember k' from Part II is "pseudo rate constant" where k' = k$[NaOH]^b$***
***Value of b and true rate constant will be determined from Part III experiments.***

# Experimental Approach (2 of 2)

## On-line Activity: Part III (Overview)

**Part III: Determine Order of [NaOH]** , i.e "b" , using the Rate law
Rate = $k[C\text{-}102]^a[NaOH]^b$
Run reaction with four different excess [NaOH] and determine k' for each run.

The k' will depend on [NaOH] depending on order with respect to

rate = $k[[\sim constant][C\text{-}102]^a = k'\,[C\text{-}102]^a$ where, **$k' = k[NaOH]^b$**

Refer to table in slide on slide 4.

*(Beware, there are few typos in Appendix A 10.2 table in lab manual.)*

***** *Remember $I/I_0$ is relative fluorescence intensity which is proportional to [C102] and that $I_0$ is the reference intensity NOT intensity at t=0)*

# Data Collection Procedure

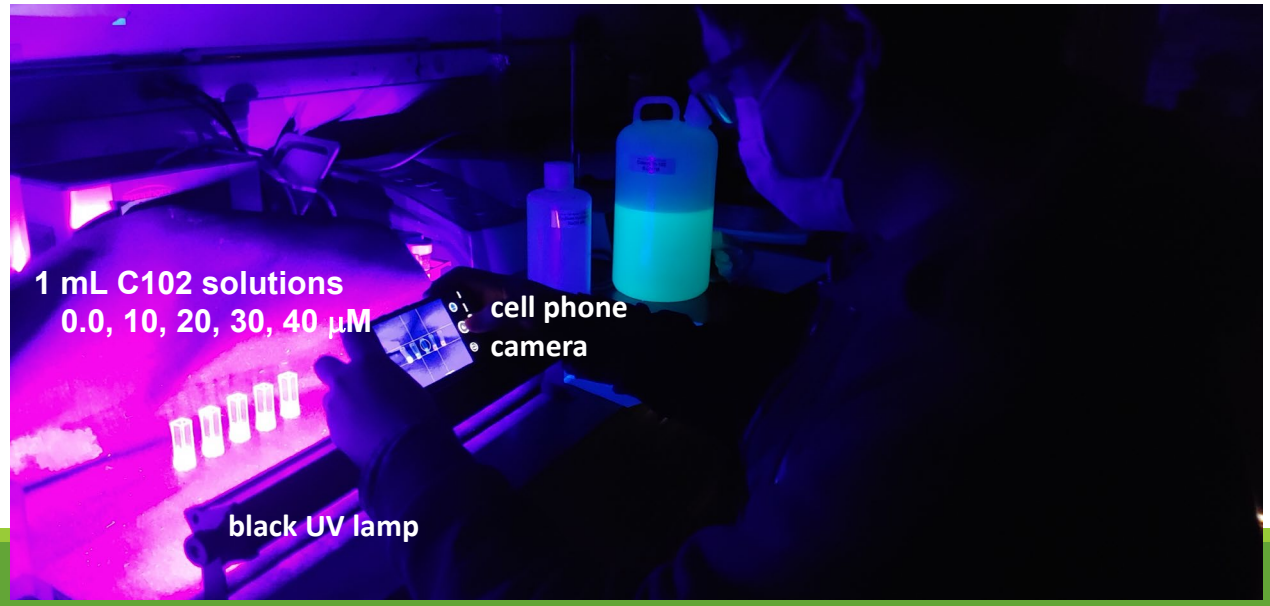
## Now we know what to do, lets get started.

**Part I: Verify C-102 Fluorescence is Proportional to Concentration**

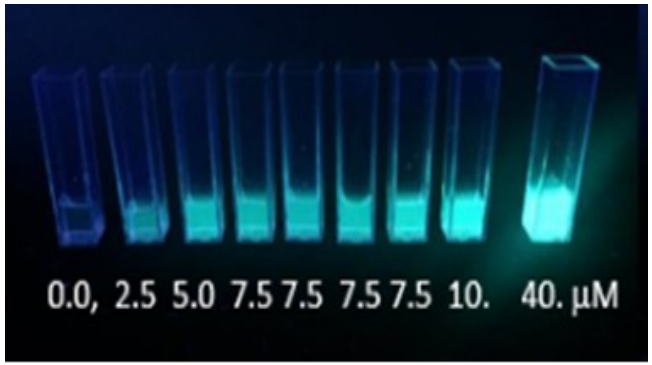
Let's grab our cuvettes, 10.0 mL volumetric flasks, pipets, and stock solutions and make series of C102 solutions for Part I.

First, fill in the table on next slide for your lab report to clearly state how you would prepare 10 mL of C102 solutions at 0.0, 10, 20, 30, 40 μM.

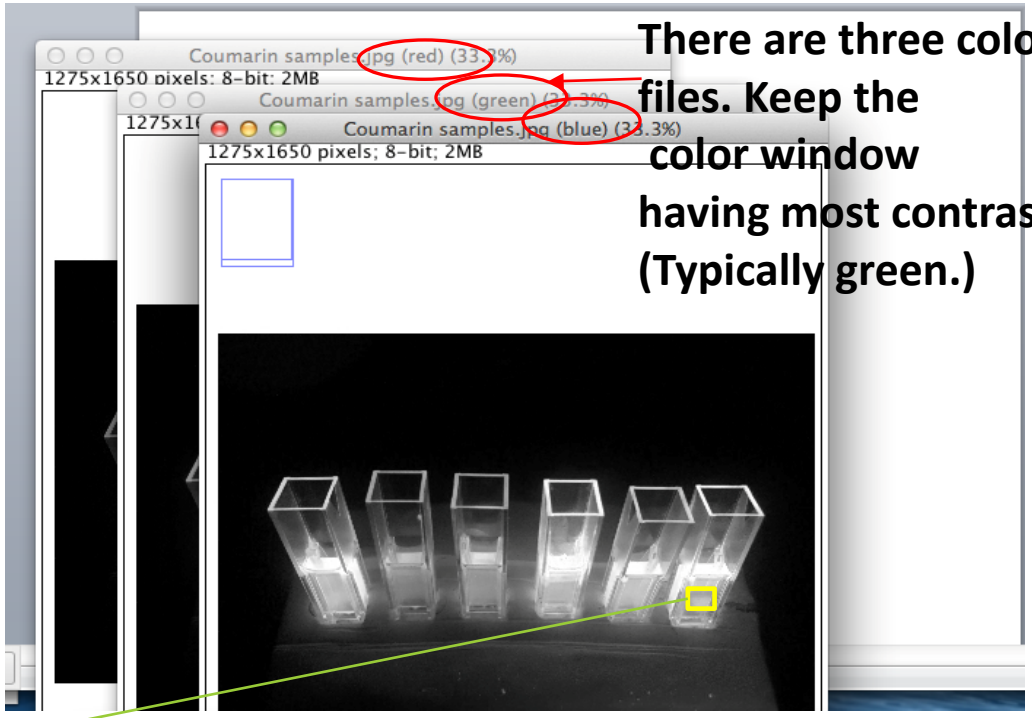
Then, 1 mL of these diluted solutions from 10 mL flasks are transferred to a row of cuvettes and irradiated evenly with a "black" UV light. The C-102 solutions fluoresce! Time to pull out your cell phone and take a snap shot of the fluorescence from all the cuvettes with UV lamp. Might try few pics with different settings on camera to maximize intensity variation. We'll use ImageJ to quantify and record intensities.



# Part I: Verify C-102 Fluorescence is Proportional to Concentration Using Image J

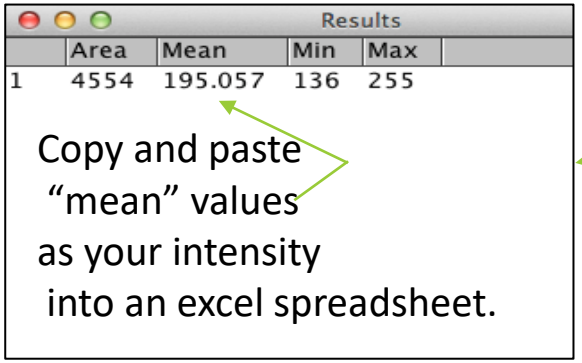



ImageJ



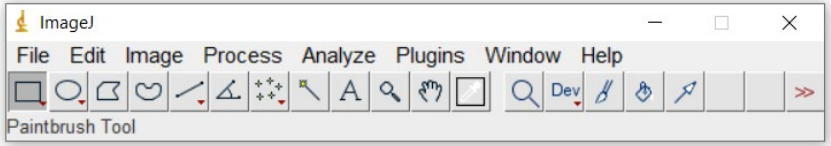

**There are three color files. Keep the color window having most contrast. (Typically green.)**

**This is just an example of [C102] 0.0 M up to 4.0 x $10^{-5}$ M (40 micromolar) done previously.**




Copy and paste "mean" values as your intensity into an excel spreadsheet.

ImageJ




***Some important pointers on using smart phone cameras with ImageJ.***

*First, whenever cuvettes are lined up to take pictures have them at least 1 cm apart.* ***Also, when analyzing with ImageJ be sure to select an area of fluorescence imaging small enough so NOT to include any edges of cuvette or top of solution as this will distort intensity.*** *And lastly, during your "photoshoots" make sure room lights are not turned off & on as a big change in ambient lights will influence fluorescence intensity results.*

***See "How to" file for further details of ImageJ.***

# Instructions for Part 1

•Prior to lab session, fill in table below by calculating volumes you would use to prepare the dilution series using the **stock solution of Coumarin-102 = 100 µM**. Complete table below for your lab report. To do this, use $M_1V_1 = M_2V_2$ to calculate volumes required for serial dilutions from stock solutions

eg: 100 µM *V1 = 50 µM * 10mL  V1 = 5 mL

•Under UV lamp illumination arrange cuvettes in increasing [C102] and take few "good" pics with cell cam. May be try different cam settings or room lights on or off.

•Use ImageJ to quantify Intensity (I) value for each concentration of C102.

*(In provisional data, for all trails, Part I, the cuvettes are arranged in increasing [C102] from left to right as: 0, 10, 20, 30, 40 µM. Choose only one trial to analyze.)*

•Use excel spreadsheet file provided to help organize your data.

Plot I vs concentration for part of lab report.

| Volume of stock Coumarin-102 (mL) | Volume of water (mL) | Total volume in cuvette | Concentration of coumarin-102 in cuvette |
|---|---|---|---|
| | | 10 mL | 0 µM |
| | | 10 mL | 10 µM |
| | | 10 mL | 20 µM |
| | | 10 mL | 30 µM |
| | | 10 mL | 40 µM |

# Lab Report Details to include for Part I

- Include brief procedure in notebook. State type of camera used and any special settings that seem to work better.

- Complete excel sheet provided (worksheet Part I) to summarize your data for concentration of C102 and fluorescence intensity.

- Make formatted plot of intensity (*I*) vs concentration ([C102]) to demonstrate the concentration dependence of fluorescence. Determine best fit line through your data.

- Attach the graph and compare experimental data with the model (goodness of fit to linear line $R^2$ i.e how close the data are to the fitted regression line)

- Analyze the results. Comment on linearity of data and if it seems to go through the origin of plot. If not, why might that be?

# Instructions for Part II

- Here we now try to determine the order of Coumarin-102 in the rate law of this rxn.
- In this case, we keep concentration of NaOH in excess so it does **NOT** change significantly during reaction.

| | Reference cuvette | Reaction cuvette |
|---|---|---|
| Volume of 100 µM C-102 stock (µL) | 100 | 100 |
| Volume of water (µL) | 900 | 600 |
| Vol. of 6M NaOH (µL) | | 300 |

- The two cuvettes need be prepared as stated above where the total solution volume in each cuvette was 1 mL.
- Take images every 30 seconds. As C-102 reacts with NaOH, the fluorescence diminishes in the reaction cuvette. Continue to take pics until intensity diminishes significantly.

*(In provisional data, you are given 13 images sequential in time inclusive of an image at t=0 s. They are listed sequentially in time left-to-right, top row-to-bottom row. In each image, cuvette on left: reference cuvette; cuvette on right: reaction cuvette. Use images from either Part II, trial 1 or Part II trial 2 if provisional data is used.)*

- Use **ImageJ** to determine $I/I_0$ for each image, remember $I_0$ is your intensity value from the reference cuvette where no reaction takes place.

Contd….

# Lab Report Details to include for Part II

- Fill out the excel sheet (worksheet, Part II) provided with the $I/I_0$ values and calculate ln $(I/ I_0)$, and $1/ (I/ I_0)$
- Plot formatted graphs of $I/I_0$ vs t , ln $(I/ I_0)$ vs t, and $[1/ (I/ I_0)]$ vs t
- Determine best $R^2$ value from the graphs which corresponds to the best fit line of data. This determines order in [C102]. (Refer to slide 5.)
- Also, the slope of the curve will provide a value for k' (rate constant). (Refer to slide 5.)
- After completing Part II, you will use these results to complete Part III.

# Instructions for Part III
## *(done on-line with image files provided)*

A series of dilutions from a 6.0 M NaOH stock solution was prepared.

They were 1.0 mL solutions prepared in test tubes at concentrations of: 0.0 M, 1.5 M, 3 M, 4.5 M, 6.0 M.

Use $M_1V_1 = M_2V_2$ to tabulate volumes of NaOH stock volumes and DI water used.

Fill in this table in notebook for part of your report.

| Test tube # (tt # in table of next slide) | Volume of stock NaOH used (µL ) | Volume of DI water (µL ) | Total volume in test tube | Concentration of NaOH in test tube |
|---|---|---|---|---|
| 1 | | | 1 mL = 1000 µL | 0.0 M |
| 2 | | | 1 mL | 1.5 M |
| 3 | | | 1 mL | 3.0 M |
| 4 | | | 1 mL | 4.5 M |
| 5 | | | 1 mL | 6.0 M |

# Instructions for Part III contd..

- Five cuvettes were collected and the following solutions shown in table below were added to make total volume of 1 mL in each cuvette.
- The solutions were added to each cuvette in the order of water, NaOH, and finally the C-102 as shown in table below.
- The solutions were placed in front of the UV light **in order of increasing [NaOH] left-to-right with cuvette on left end having 0.0 M NaOH whose fluorescence will serve as your reference ($I_0$).**
- Images of all five cuvettes were obtained every 30 seconds starting at t=0.0 s and are listed sequential in time left-to-right, top row-to-bottom row. As C-102 reacts with NaOH, the fluorescence diminishes in each reaction cuvette containing NaOH (#2-5).

| Cuvette # (Left to right in images provided) | Volume of water (µL) | Volume of NaOH (µL) [from test tubes (tt #1-5) of varying dilutions in table on slide 12.] | Volume of C-102 from stock solution (µL) | Total volume (µL) |
|---|---|---|---|---|
| 1 | 600 | 300 from tt #1 | 100 | 1000 |
| 2 | 600 | 300 from tt #2 | 100 | 1000 |
| 3 | 600 | 300 from tt #3 | 100 | 1000 |
| 4 | 600 | 300 from tt #4 | 100 | 1000 |
| 5 | 600 | 300 from tt #5 | 100 | 1000 |

# What is the $NaOH_{eff}$ after second dilution from table on slide 13 ?

**Show in your lab report the calculation of the $[NaOH]_{eff}$ for each of the cuvettes (#2-5), it is not 1.5M, 3 M etc..**

**Refer to further information below.**

Recall, we prepared 1 mL solutions of varying [NaOH] in test tubes but only used 0.3 mL (300 µL) of the test tube solutions to make 1 mL of solutions in reaction cuvettes. **This leads to a second dilution.**

Use $M_1 V_1 = M_2 V_2$ to determine the $[NaOH]_{eff}$, in each reaction cuvette prepared in part III

eg: 1.5 M*300 µL = $\mathbf{M_2}$ * 1000 µL

# Summary Table of $[NaOH]_{eff}$, $[NaOH]_{eff}^2$ and k'

| $[NaOH_{eff}]$ | $[NaOH_{eff}]^2$ | k' |
|---|---|---|
| | | |
| | | |
| | | |
| | | |

Include this table as part of your report. You will need to make plots of these data.

# Lab Report Details for Part III

- **Fill in table from slide 13 and include it in your report.**
- **Use imageJ to analyze the Part III image files provided for you. Complete excel sheet for $I/I_0$ for the different $[NaOH]_{eff}$. See excel file, worksheet Part III.**

***(In Part III provisional data, cuvette #1 has 0.0 M NaOH. (on left end) in image file is $I_0$ (reference intensity)***

- **You first plot four graphs (one for each non-zero NaOH concentration) using best linear graph according to instructions from Part II (slide 12). Note this requires results from Part II to identify order of reaction in C-102. But by this point most of Part III work is done. )**
- **The slope of each graph are your four k' values corresponding to the four non-zero concentrations of NaOH**
- **Complete Summary Table on slide 16 in preparation of making two more graphs below.**
- **So in this case,**
- **$k' = k[NaOH]^b$ you will assume b= 1 and b=2 and plot two more graphs of**
  - **k' vs $[NaOH]_{eff}$ k' vs $[NaOH]_{eff}^2$ (Refer to Summary Table, slide 16.)**

**•The highest $R^2$ value will enable you to determine if k' vs $[NaOH]_{eff}$ or k' vs $[NaOH]_{eff}^2$ graph gives better relationship between the variables.**

**•This will determine the order with respect to [NaOH] in the rate law.**

# Instructions for post lab report in a nutshell

- Write up lab notebook pages for header information and pre-lab procedure as if you were to do experiment in lab.
- Summarize Observation of Data, Calculations, Results as follows:
- Part I: Plot of I vs [C-102], discuss about linearity of the curve, make use of $R^2$ value, have a table of data along with the graph for the points you plot
- Part II: Fill up excel sheet using 13 images from Part II trial 1 or 2. Plot three graphs to determine order with respect to [C-102]. The graphs must be $I/I_0$ vs t, ln $I/I_0$ vs t and 1/ $I/I_0$ vs t.
- Make sure all plots are fully formatted with chart titles, axis titles with units etc.
- Part III: After analyzing Part III image files with ImageJ and determining [NaOH]eff (slide xx) choose form of graph which is most linear from Part II to make same type of plot and determine k' for each [NaOH]
- For example if it's $I/I_0$ vs t, use this plot type for all the four cuvette images. Choose one set of image files labelled as Part III, trials 1, 2, or 3.
- Determine k' values from each of these graphs
- Provide Summary Report (slide 16) and plot 2 more graphs of k' vs $[NaOH]_{eff}$ and k' vs $[NaOH]_{eff}^2$ and determine order with respect to [NaOH].
- Finally, write the complete rate law giving order with respect to [C102] and [NaOH].
- ***Reach out to either your TA or any other Chem125 TA in MSLC as needed.***

*****There is a lot of data to analyze in this expt so the sooner you get started the better.*****

# Post Lab Report Submission:

Go to Labflow Lab 6 Postlab → → Upload as single pdf file.

In submitting report combine all notebook page images, data tables, and graphs (as needed) into one pdf file. **This is most easily done by copy and pasting all images, tables, and graphs into one Word document and then save file as a pdf document.**
Make sure all images are presented in an organized manner prior to uploading with each part of experiment clearly labelled.

Each TA may have more specific submission instructions for this lab experiment.

**Lab report submission for this experiment (<u>Parts I, II, and III</u>) for both Lab Report for all sections due 1 hr before lab session of week 15.**

***This lab include in-lab experiments (Parts I and II) with 20 pts and an on-line activity (Part III) worth 10 pts. So entire lab report worth 30 pts.***